



P.E. 5818 - Patentaan 2
2280 HV Rijswijk (ZH)
☎ (070) 3 40 20 40
FAX (070) 3 40 30 16

Europäisches
Patentamt

European
Patent Office

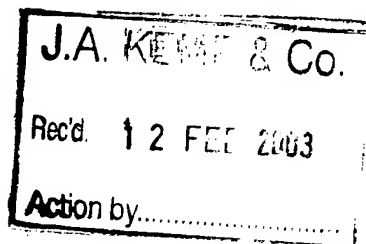
Office européen
des brevets

Generaldirektion 1

Directorate General 1

Direction générale 1

Cresswell, Thomas Anthony
J.A. KEMP & CO.
14 South Square
Gray's Inn
London WC1R 5JJ
GRANDE BRETAGNE



epoline® Customer Services

Tel.: +31 (0)70 340 45 00

Date
10.02.03

Reference N.84373	Application No./Patent No. 01310251.2 - 2405
Applicant/Proprietor Sumitomo Chemical Company, Limited	

**Communication pursuant to Rule 50 EPC - reminder of payment of the designation fees
(Art. 79(2) EPC) and of the examination fee (Art. 94(2) EPC)**

The date on which the European Patent Bulletin mentions the publication of the European search report for the above-mentioned European patent application is: 05.02.03.

Your attention is drawn to Article 79(2) and (3) EPC as well as Article 94(2) and (3) EPC according to which within **SIX MONTHS** after the above-mentioned publication date of the search report

- the designation fee(s) must be paid,
- a written request for examination must be filed as well as the examination fee must be paid.
(A written request for examination has been filed already.)

The current rate of the designation fee for each contracting state designated is: EUR 75,00

If the application has been filed on or after 01 July 1999 the payment of seven times the amount of the designation fee is deemed to constitute payment of the designation fees for all contracting states (see OJ EPO 06/1999, 405).

The current rate of the examination fee is: EUR 1430,00

If at least one designation fee and the examination fee are not paid within the period laid down in Article 79(2) or 94(2) EPC, the application shall be deemed to be withdrawn (Arts. 79(3), 94(3) EPC).

Any extension fees are also payable within the above-mentioned period.

**NOTE TO USERS OF THE AUTOMATIC DEBITING PROCEDURE:****1) Designation fees**

If the application has been filed up to 30 June 1999, the designation fees for the contracting states marked under no. 2 of section 32 of the Request for Grant (EPO Form 1001) will be debited on the last day of the period pursuant to Article 79(2) EPC, unless the EPO receives prior instructions to the contrary.

If the application has been filed on or after 01 July 1999, seven times the amount of the designation fee will be debited on the last day of the period pursuant to Article 79(2) EPC. However, if contracting states are marked under no. 2 of section 32 of the Request for Grant (EPO Form 1001), the designation fees only for these contracting states will be debited unless instructions to the contrary have reached the EPO within the basic period for paying the designation fees.

2) Examination fee

Unless the EPO receives prior instructions to the contrary, the examination fee will be debited on the last day of the period for payment.

For further details see the Arrangements for the automatic debiting procedure, Supplement to OJ EPO 02/2002.

RECEIVING SECTION

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 1 213 354 A3

(12)

EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
05.02.2003 Bulletin 2003/06

(51) Int Cl.7: **C12N 15/53**, C12N 9/04,
C12N 1/21, C12P 7/42,
C12P 7/62

(43) Date of publication A2:
12.06.2002 Bulletin 2002/24

(21) Application number: **01310251.2**

(22) Date of filing: **07.12.2001**

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE TR**
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **07.12.2000 JP 2000372704**
15.01.2001 JP 2001006144
02.02.2001 JP 2001026594
11.06.2001 JP 2001175175

(83) Declaration under Rule 28(4) EPC (expert
solution)

(71) Applicant: **Sumitomo Chemical Company,
Limited**
Chuo-ku Osaka 541-8550 (JP)

(72) Inventors:

- **Asako, Hiroyuki**
Minoo-shi, Osaka (JP)
- **Matsumura, Kenji**
Minoo-shi, Osaka (JP)
- **Shimizu, Masatoshi**
Toyonaka-shi, Osaka (JP)
- **Ito, Nobuya**
Tayama-shi, Toyama (JP)
- **Wakita, Ryuhei**
Toyonaka-shi, Osaka (JP)

(74) Representative: **Cresswell, Thomas Anthony**
J.A. KEMP & CO.
14 South Square
Gray's Inn
London WC1R 5JJ (GB)

(54) **Process for producing optically active 4-halo-3-hydroxybutanoate**

(57) There are provided a polynucleotide sequence coding for an amino acid sequence capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate, a DNA construct having a promoter in operative linkage

with the polynucleotide sequence, a recombinant vector comprising the polynucleotide sequence, a transformant, a recombinant vector and the like.

EP 1 213 354 A3



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 01 31 0251

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	EP 0 967 271 A (KANEGAFUCHI CHEMICAL IND) 29 December 1999 (1999-12-29)	1-19	C12N15/53 C12N9/04
Y	* the whole document *	1-23, 30-37	C12N1/21 C12P7/42 C12P7/62
X	EP 1 013 758 A (DAICEL CHEM) 28 June 2000 (2000-06-28)	1-19	
Y	* the whole document *		
Y	EP 0 606 899 A (DAICEL CHEM) 20 July 1994 (1994-07-20)	1-22	
Y	* the whole document *		
X	WO 00 71503 A (UNIV MICHIGAN) 30 November 2000 (2000-11-30)	24-29	
Y	* page 10, line 11 - page 11, line 16 *	30-37	
Y.D	WANG J -C ET AL: "Cloning, sequence analysis, and expression in Escherichia coli of the gene encoding phenylacetaldehyde reductase from styrene-assimilating Corynebacterium sp. strain ST-10." APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 52, no. 3, 1999, pages 386-392, XP002221908 ISSN: 0175-7598	23	
	* the whole document *		
A	WO 99 42590 A (BIRCH OLWEN ;KIENER ANDREAS (CH); LONZA AG (CH); THOENI SUSANNE (C) 26 August 1999 (1999-08-26)		
The present search report has been drawn up for all claims			
Place of search		Date of completion of the search	Examiner
MUNICH		21 November 2002	Kania, T
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document</p> <p>T: theory or principle underlying the invention I: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons A: member of the same patent family, corresponding document</p>			



European Patent
Office

Application Number

EP 01 31 0251

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s).
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims.



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 01 31 0251

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	KATAOKA M ET AL: "Stereoselective reduction of ethyl 4-chloro-3-oxobutanoate by Escherichia coli transformant cells coexpressing the aldehyde reductase and glucose dehydrogenase genes." APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 51, no. 4, April 1999 (1999-04), pages 486-490, XP002209640 ISSN: 0175-7598		
A	WO 93 18138 A (KERNFORSCHUNGSANLAGE JUELICH) 16 September 1993 (1993-09-16)		
A	DATABASE EMBL 'Online! 12 October 1998 (1998-10-12) KIMURA M.: "Gibberella zeae gene for reductase" retrieved from EBI Database accession no. AC014493; 074646 XP002209648 * 77% identity to SEQ ID NO:2; 73% identity to SEQ ID NO:1 * * abstract *		TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	DATABASE WPI Week 198546, 1985 Derwent Publications Ltd., London, GB; AN 1985-287883 XP002209642 & JP 60 199393 A (SUMITOMO CHEM IND KK), 8 October 1985 (1985-10-08) * abstract *	20,22 Amended cited be correct	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	21 November 2002	Kania, T
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background U: non-written disclosure P: intermediate document</p> <p>1: theory or principle underlying the invention 2: earlier patent document, but published on, or after the filing date 3: document cited in the application 4: document cited for other reasons 5: member of the same patent family corresponding document</p>		



European Patent
Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 01 31 0251

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-22 completely

A polynucleotide according to SEQ ID NO:2 encoding a polypeptide according to SEQ ID NO:1 preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate, and related subject-matter. A process for producing (S)-4-halo-3-hydroxybutanoate using said polypeptide.

A process for producing an optically active 3-hydroxybutanoic acid ester employing a microorganism belonging to *Penicillium citrinum*, especially *P. citrinum* IF04631.

A process for producing an optically active 3-hydroxybutanoic acid ester employing a microorganism belonging to *Cryptococcus humicolus*, especially *C. humicolus* IF01527.

A process for producing an optically active 3-hydroxybutanoic acid ester employing a microorganism belonging to *Bacillus alvei*, especially *B. alvei* IF03343t.

1.1. Claims: 1-19 completely, 20-22 partially

A polynucleotide according to SEQ ID NO:2 encoding a polypeptide according to SEQ ID NO:1 preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate, and related subject-matter. A process for producing (S)-4-halo-3-hydroxybutanoate using said polypeptide. A process for producing an optically active 3-hydroxybutanoic acid ester employing a microorganism belonging to *Penicillium citrinum*, especially *P. citrinum* IF04631.

1.2. Claims: 20-22 partially

A process for producing an optically active 3-hydroxybutanoic acid ester employing a microorganism belonging to *Cryptococcus humicolus*, especially *C. humicolus* IF01527.

1.3. Claims: 20-22 partially

A process for producing an optically active 3-hydroxybutanoic acid ester employing a microorganism belonging to *Bacillus alvei*, especially *B. alvei* IF03343t.



European Patent
Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 01 31 0251

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

2. Claim : 23 completely

A process for producing an optically active 4-bromo-3-hydroxybutanoate by reacting the substrate with an enzyme having the amino acid sequence according to SEQ ID NO:34, encoded by the polynucleotide of SEQ ID NO:35, and related subject-matter.

3. Claims: 24-37 completely

A process for producing 4-cyano-3-hydroxybutanoic acid or an ester thereof by chemical synthesis, or conducting the asymmetrical reduction reaction by a microorganism belonging to *Penicillium citrinum*, especially *P. citrinum* IF04631, and related subject-matter.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 01 31 0251

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	<p>DATABASE CA Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; TAKAHASHI, HIDEYUKI ET AL: "Microbial production of D-.alpha.-amino acids" retrieved from STN Database accession no. 107:76090 XP002209641 * abstract *</p> <p>& JP 62 025990 A (KANEKAFUCHI CHEMICAL INDUSTRY CO., LTD., JAPAN) 3 February 1987 (1987-02-03)</p>	20,22	<p>TECHNICAL FIELDS SEARCHED (Int.Cl.7)</p>
A	<p>ITOH NOBUYA ET AL: "Production of chiral alcohols by enantioselective reduction with NADH-dependent phenylacetaldehyde reductase from Corynebacterium strain, ST-10." JOURNAL OF MOLECULAR CATALYSIS B ENZYMATIC, vol. 6, no. 1-2, 4 January 1999 (1999-01-04), pages 41-50, XP002221909 ISSN: 1381-1177 * the whole document *</p>	23	
A	<p>ITOH NOBUYA ET AL: "Purification and characterization of phenylacetaldehyde reductase from a styrene-assimilating Corynebacterium strain, ST-10." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 63, no. 10, 1997, pages 3783-3788, XP002221910 ISSN: 0099-2240</p>	23	
The present search report has been drawn up for all claims			
Place (search)		Date of completion of the search	Examiner
MUNICH		21 November 2002	Kania, T
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X particularly relevant if taken alone Y particularly relevant if combined with another document of the same category A technological background O non-written disclosure P intermediate document</p> <p>I theory or principle underlying the invention E earlier patent document, but published on, or after the filing date D document cited in the application L document cited for other reasons & member of the same patent family, corresponding document</p>			

EPOFORM 1503 02 92 (PatCO1)



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 01 31 0251

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	TROOSTWIJK C B ET AL: "METHOD FOR THE SYNTHESIS OF 4-SUBSTITUTED ACETOACETATES" JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS, CHEMICAL SOCIETY, LETCHWORTH, GB, no. 23, 7 December 1977 (1977-12-07), pages 932-933, XP000571420 ISSN: 0022-4936 * the whole document *	24-37	
A	US 4 978 768 A (VON DER CRONE JOST ET AL) 18 December 1990 (1990-12-18) * the whole document *	25, 27, 36, 37	
E	EP 1 201 647 A (SUMITOMO CHEMICAL CO) 2 May 2002 (2002-05-02) * the whole document *	23	
T	ITOH NOBUYA ET AL: "Chiral alcohol production by NADH-dependent phenylacetaldehyde reductase coupled with in situ regeneration of NADH." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 269, no. 9, May 2002 (2002-05), pages 2394-2402, XP002221911 =ejb&page=aims May, 2002 ISSN: 0014-2956	23	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 21 November 2002	Examiner Kania, T
CATEGORY OF CITED DOCUMENTS		1. theory or principle underlying the invention E. earlier patent document, but published on, or after the filing date D. document cited in the application L. document cited for other reasons A. member of the same patent family corresponding document X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background U: non-written disclosure P: intermediate document	

11/03/03 26:00:05: MWJ/CJ3

ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.

EP 01 31 0251

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

21-11-2002

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
EP 0967271	A	29-12-1999	AU	4032997 A	26-08-1998
			EP	0967271 A1	29-12-1999
			SI	20120 A	30-06-2000
			SK	105699 A3	12-06-2000
			US	6218156 B1	17-04-2001
			WO	9835025 A1	13-08-1998
			US	2002006651 A1	17-01-2002
EP 1013758	A	28-06-2000	JP	2000236883 A	05-09-2000
			EP	1013758 A2	28-06-2000
			US	6312933 B1	06-11-2001
			US	2002042110 A1	11-04-2002
			US	2002127679 A1	12-09-2002
EP 0606899	A	20-07-1994	JP	3155107 B2	09-04-2001
			JP	6209782 A	02-08-1994
			CA	2113240 A1	13-07-1994
			DE	69422064 D1	20-01-2000
			DE	69422064 T2	25-05-2000
			EP	0606899 A2	20-07-1994
			US	5559030 A	24-09-1996
WO 0071503	A	30-11-2000	US	6114566 A	05-09-2000
			AU	4662900 A	12-12-2000
			EP	1180095 A1	20-02-2002
			WO	0071503 A1	30-11-2000
WO 9942590	A	26-08-1999	AU	2926599 A	06-09-1999
			CA	2311649 A1	26-08-1999
			WO	9942590 A1	26-08-1999
			EP	1054974 A1	29-11-2000
			JP	2002504337 T	12-02-2002
WO 9318138	A	16-09-1993	CA	2117482 A1	16-09-1993
			WO	9318138 A1	16-09-1993
			DE	59306681 D1	10-07-1997
			DK	630402 T3	22-12-1997
			EP	0630402 A1	28-12-1994
			JP	7505770 T	29-06-1995
			US	5523223 A	04-06-1996
JP 60199393	A	08-10-1985	JP	1822917 C	10-02-1994
			JP	5032035 B	14-05-1993
JP 62025990	A	03-02-1987	JP	1048758 B	20-10-1989
			JP	1561892 C	31-05-1990

EPO FORM P0455

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 01 31 0251

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on:
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

21-11-2002

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
US 4978768	A	18-12-1990	CH	664954 A5	15-04-1988
			DE	3700675 A1	16-07-1987
			FR	2601005 A1	08-01-1988
			GB	2185482 A .B	22-07-1987
			IT	1201152 B	27-01-1989
			JP	1985107 C	25-10-1995
			JP	7023351 B	15-03-1995
			JP	62169755 A	25-07-1987
			KR	9408916 B1	28-09-1994
EP 1201647	A	02-05-2002	EP	1201647 A2	02-05-2002
			JP	2002201169 A	16-07-2002
KR 2000015622	A	15-03-2000	NONE		

EPO FORM 02/99

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82